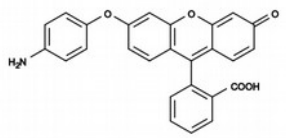
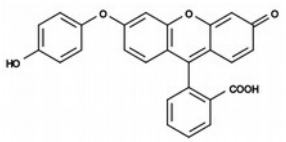
FT-AZ9811

APF

Optimized for Detecting Reactive Oxygen Species (ROS)

Product name cat.number	MW (g·mol-1)	λ_{exc}\λ_{em}. max. (nm)	Structure
APF FP-AZ9811, 1mg	423.42	490 / 515	
HPF FP-AZ980A, 1mg	424.40	490 / 515	

Storage: -20°C Protect from light and moisture

Introduction

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include superoxide, hydroxyl radical, singlet oxygen and peroxides. ROS is highly reactive due to the presence of unpaired valence shell electrons. ROS forms as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation. Under conditions of oxidative stress, ROS production is dramatically increased, resulting in subsequent alteration of membrane lipids, proteins, and nucleic acids. Oxidative damage of these biomolecules is associated with aging as well as with a variety of pathological events, including atherosclerosis, carcinogenesis, ischemic reperfusion injury, and neurodegenerative disorders. APF is a fluorogenic probe to measure hydroxyly radical in cells using conventional fluorescence microscopy, high-content imaging, microplate fluorometry, or flow cytometry. The cell-permeant APF reagent is nonfluorescent and produces bright green fluorescence upon reaction with hydroxyl radical. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. In the presence of peroxidase, APF also reacts with hydrogen peroxide. APF has good selectivity to hydroxyl radical compared to other ROS. APF and HPF show relatively high resistance to light-induced oxidation. APF and HPF are nonfluorescent until they react with the hydroxyl radical or peroxynitrite anion. APF will also react with the hypochlorite anion.

Directions for use

Fluorescent staining for adherent cells

This protocol only provides a guideline, and should be modified according to your specific needs. Treat cells as desired before making the working solution.

1. Prepare a 10 to 20 mM APF (or HPF) stock solution in DMSO. Make 1 to 10 µM working solution by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).
2. Treat cells as desired (e.g., RASM cells are treated with 50-100 nM angiotensin II for 3-5 hours)
3. Incubate the cells with APF (or HPF) (1-10 µM, from Step #1) for 20 -60 minutes at 37ºC.
4. Replace the dye-loading solution with HHBS buffer.
5. Analyze the cells with a proper fluorescence instrument at Ex/Em = 490/525 mm (cut off = 515 nM) with bottom read mode (e.g., FITC filter set for a fluorescence microscope, FL1 filter for a flow cytometer).

Note: BSA and phenol red can affect the fluorescence and should be used with caution. Both APF and HPF can be used in solution assays or for intracellular measurements.

References

- **Du M**. *et al.*, Developmental toxicity evaluation of three hexabromocyclododecane diastereoisomers on zebrafish embryos, *Aquat Toxicol*, 112-113, 1 (2012)
- **Park WH**. *et al.*, MAPK inhibitors and siRNAs differentially affect cell death and ROS levels in arsenic trioxide-treated human pulmonary fibroblast cells. *Oncol Rep*, 27, 1611 (2012)

Related products

Dihydrorhodamine 123, FP-83775A

H2DCFDA, FP-467312

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com.
Please inquire for higher quantities (availability, shipment conditions).
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